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## ARBUSCULAR MYCORRHIZA OF PLANTS SPONTANEOUSLY COLONIZING THE SODA HEAP IN JAWORZNO (SOUTHERN POLAND)

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### ABSTRACT

The results of studies of the mycorrhizal status of plant species spontaneously established on the soda heap located in Jaworzno (Upper Silesia, Poland) are presented. Additionally, the species of arbuscular fungi of the phylum Glomeromycota extracted from field-collected rhizosphere substrate samples of the heap are showed. Arbuscular mycorrhizae were described in 17 plant species. Five *Glomus* spp. were recognized in the spore populations of arbuscular fungi isolated. The investigation presented in this paper for the first time revealed *Centaurea stoebe* and *Trifolium montanum* to be hosts of arbuscular fungi.

**KEY WORDS:** arbuscular mycorrhiza, industrial wastes, soda heap, Glomeromycota.

### INTRODUCTION

Massive destruction of native vegetation occur throughout the world because of the industrial land use by people. Top soil removal and piling up heaps usually highly reduce the occurrence and activity of soil microorganisms beneficial for plants, among which are arbuscular mycorrhizal fungi (AMF) of the phylum Glomeromycota (Jasper et al. 1987; Rives et al. 1980; Stahl et al. 1988; Bellgard 1993) suggested to co-exist in an obligate symbiosis with at least 80% of all plants of the world (Gianinazzi and Gianinazzi-Pearson 1986).

Arbuscular mycorrhizae (AM) alleviate many anthropogenic stresses, including those caused by heavy metals and toxic inorganic and organic substances deposited in the soil, as well as those resulting from too high compaction and pH of soil (Schüepp et al. 1987; Gildon and Tinker 1983; Weissenhorn et al. 1995; Entry et al. 2002). Additionally, AMF improve natural succession of plants in habitats where their colonization is slow, e.g., on sand dunes (Sylvia and Will 1988; Corkidi and Rincon 1997a, b) and contaminated areas such as industrial wastes (Khan 1978; Hetrick et al. 1994; Gucwa-Przepióra and Turnau 2001).

Many post-industrial sites have been recognized as refuges for protected and rare plant species, including the soda heap at Jaworzno (Upper Silesia, Poland; Cohn et al. 2001). Thus, considering the positive results of the co-occurrence of plants and AMF presented above, the reco-

gnition of the mycorrhizal status of plants and associated AMF in such sites may be helpful in the elaboration of strategies of protection of these associations by e.g., introduction of species of AMF best adapted to particular local conditions.

The main aim of the study presented here was to assess the mycorrhizal colonization of selected herbaceous plants spontaneously established in an old solvay process heap in Jaworzno. Additionally, this paper shows species of the mycorrhizal fungi extracted from field-collected substrate samples of the heap.

### MATERIALS AND METHODS

#### *Site description*

The study area was located in Jaworzno-Szczakowa, which lies in the Jaworzno Hills in the Silesian Upland (50°18'N; 19°10'E). The heap is situated close to the Szczakowa window glass factory and Katowice–Cracow railway tracks. The site occupies ca. 2 ha and is isolated on all sides by steep, more or less bare, vertical eroded banks, ca. 3 m high (Fig. 1). It is an abandoned solvay process slurry heap from a former Austrian soda factory, which produced soda until 1911. Thus, the present day heap probably represents the place where calcium chloride was deposited.

The vegetation of the study area mainly consisted of species characteristic for meadows and grasslands. Additio-



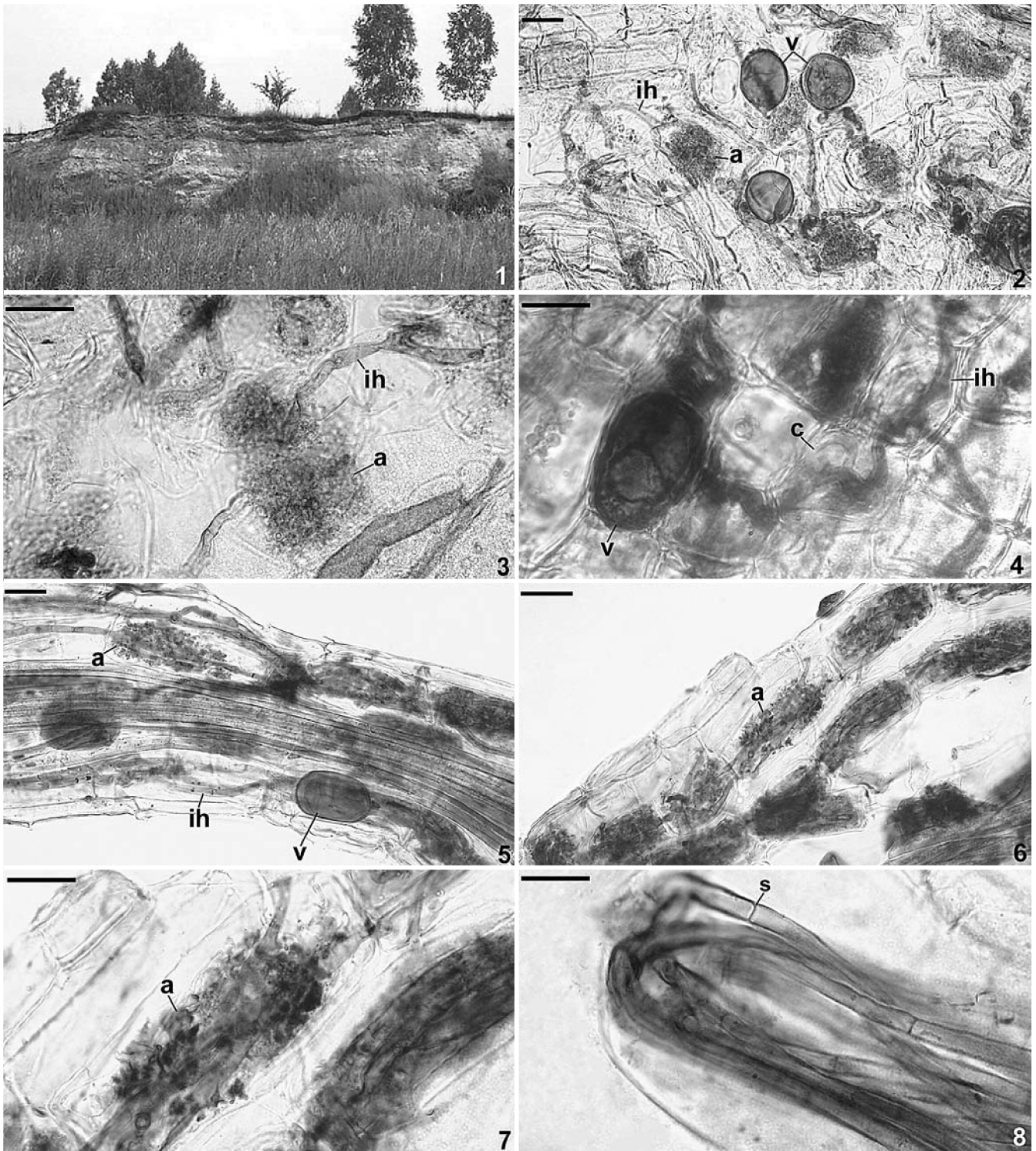


Fig. 1. General view of the solvay process heap in Jaworzno. Figs 2 and 3. Arbuscules (a), vesicles (v), and intraradical hyphae (ih) in roots of *Centaurea stoebe*. Fig. 4. Vesicle and coil in a root of *Tofieldia calyculata*. Figs 5-7. Arbuscules (a), vesicles (v), and intraradical hyphae (ih) in roots of *Trifolium montanum*. Fig. 8. Coil in a root of *Epipactis atrorubens*; septum (s) is indicated. Bars: Figs 2, 5, and 6=20  $\mu$ m; Figs 3, 7, and 8=10  $\mu$ m.

nally, birch trees and shrubs occasionally occurred there. The site is shown to be an important refuge for some protected and mountain species, and other elements uncommon in the local flora (Cohn et al. 2001).

#### Substrate

The solvay process produced "white seas" of calcium chloride as an aqueous suspension, so the site is the place where it was probably deposited. Previous investigations showed that the external layer of the heap consists of slag

and soil mixture (unpublished material gathered by the existing window glass factory Szczakowa SA). The deeper layer contains lime waste with a significant proportion of magnesium and a low level of chloride ions.

Properties of the substrate were analyzed as follows: total N was determined with the Kjeldahl method, organic C with the Tiurin method, available P with the Egner-Rhiem method, pH and EC were measured in water extract with combination glass/calomel electrode and pH meter. General properties and the concentration of elements of the

TABLE 1. General properties of the Jaworzno soda heap substrate.

pH (H <sub>2</sub> O)	7.7
N total [g kg <sup>-1</sup> air d.w.]	4.06
C organic [g kg <sup>-1</sup> air d.w.]	87.14
P available [mg kg <sup>-1</sup> air d.w.]	8.38
C:N	22.33
EC [μS/cm]	351.5

TABLE 2. Concentration of elements in the substrate. Values represent range of elements concentration as percentage of sieved, dry soil (X-Ray Fluorescence Spectrometry) (Cohn et al. 2001).

Al	4.59-8.85
Ba	0.034-0.081
Ca	24.19-39.47
Cl	0.12-0.27
Cu	0.003-0.006
Fe	2.20-5.71
K	0.41-0.95
Mg	0.59-2.09
Mn	0.034-0.75
Na	0.25-0.34
P	0.16-0.21
Pb	0.21-0.036
S	0.23-0.40
Si	17.41-29.32
Ti	0.15-0.39
Zn	0.10-0.20

Jaworzno soda heap rhizosphere substrate are listed in Table 1 and Table 2.

#### *Sampling of plants, roots, and assessment of AM colonization*

Specimens of twenty common herbaceous plant species spontaneously established in the Jaworzno soda heap were collected in July 2004. Latin names of the plants follow Mirek et al. 2002. The plant specimens were deposited in the herbarium of the University of Silesia (KTU).

To evaluate AM colonization, root samples of at least five flowering plants of each species were collected from a depth of 0-20 cm. After their transfer to a laboratory, the roots were first washed out in tap water, then cut into 1.0-cm-long segments, softened in 7% KOH at 60°C for 1 h, rinsed in a few changes of water, acidified in 5% lactic acid at room temperature for 1-24 h, and finally stained with 0.01% aniline blue in 5% lactic acid at 60°C for 0.5 h. The stained root segments were stored in lactoglycerol until they were used for slide preparation.

Mycorrhizal colonization of the plant species sampled was assessed following the calculation of mycorrhizal frequency (F%), intensity of root cortex colonization (M%), and arbuscule abundance (A%) according to the method described by Trouvelot et al. (1986; <http://www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html>).

#### *Collection of rhizosphere substrates, isolation and identification of AMF*

To determine species of arbuscular fungi occurring in the heap examined, during flowering of its most plants in July

2005, rhizosphere substrate were collected in 3 randomly selected sites from a depth of 5-20 cm using a small garden shovel. About 500 cm<sup>3</sup> samples were placed in plastic bags. In the laboratory, they were first air dried and then stored at 4°C for ca. one month until processing.

Spores of AMF were extracted by wet sieving and decanting (Gerdemann and Nicolson 1963). Morphological properties of spores and their subcellular structures were determined based on at least 30 spores mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG; Omar et al. 1979) and a mixture of PVLG and Melzer's reagent (1:1, v/v). Spores were crushed to varying degrees by applying pressure to the cover slip and then stored at 65°C for 24 h to clear their contents from oil droplets. These were examined under an Olympus BX 50 compound microscope equipped with Nomarski differential interference contrast optics. Microphotographs were recorded on a Sony 3CDD color video camera coupled to the microscope. Vouchers of all the fungal species recovered are preserved in the authors' collections. Colour microphotographs of spores and mycorrhizae of the AM fungal species found can be viewed at the URL <http://www.agro.ar.szczecin.pl/~jblaszkowski/>.

## RESULTS

The mycorrhizal status of vegetation of the soda heap in Jaworzno was determined based on root samples of 20 species belonging to 12 plant families. Arbuscular mycorrhizae were found in 17 plant species. The mycorrhizal structures of all the plant species found to host arbuscular fungi comprised arbuscules, and vesicles (Table 3; Figs 2, 3 and 5-7), with the exception of *Anthyllis vulneraria*, which mycorrhizal roots did not contain vesicles as well as coils (Table 3). Coils were present among arbuscular mycorrhizae of 8 plant species. Few coils and vesicles also occurred in roots of *Tofieldia calyculata*, although no arbuscules accompanied them (Table 3, Fig. 4).

Except for *Euphrasia stricta* having a relatively low AM frequency, the average frequencies of AM colonization in the other plant species ranged from 80 to 100% (Table 3).

In 13 plant species, more than 50% of their root cortex was colonized by AMF (Table 3). The intensity of root cortex colonization (M%) in the other four plant species was mean and ranged from 15.7% (*Euphrasia stricta*) to 45.5% (*Dianthus carthusianorum*).

The arbuscule abundance (A%) exceeded 50% in *Achillea millefolium*, *Molinia coerulea*, *Plantago media*, and *Prunella vulgaris* (Table 3). In 12 plant species, it was within the 10-50% range. Low relative arbuscular richness was found only in *Euphrasia stricta* (4.1%).

In the field-collected substrate samples, a total of five species of AMF were identified. All represented the genus *Glomus*. Only spores of *G. fasciculatum* and *G. mosseae* were not numerous (<50 spores in 100 g dry soil). The spore abundance of the other species was high (50-100 spores in 100 g dry soil; *G. macrocarpum*) to very high (>100 spores in 100 g dry soil; *G. constrictum* and *G. microcarpum*).

Mycorrhizal status of two representatives of the family Orchidaceae: *Epipactis atrorubens* and *Epipactis palustris* were also examined, but only the former one harboured orchid mycorrhizae (Fig. 8).



TABLE 3. AM structures and AM colonization of plants from the soda heap in Jaworzno (values listed as means  $\pm$  standard errors).

Plant species	AM structures			AM colonization		
	A	V	C	F%	M%	A%
<i>Achillea millefolium</i>	+	+	–	100 $\pm$ 0	87.3 $\pm$ 5.4	80.0 $\pm$ 3.3
<i>Anthyllis vulneraria</i>	+	–	–	100 $\pm$ 0	70.2 $\pm$ 3.8	36.0 $\pm$ 4.5
<i>Asperula cynanchica</i>	+	+	+	100 $\pm$ 0	57.3 $\pm$ 4.1	46.5 $\pm$ 6.0
<i>Briza media</i>	+	+	+	100 $\pm$ 0	74.7 $\pm$ 3.9	43.7 $\pm$ 4.3
<i>Calamagrostis epigeios</i>	+	+	–	100 $\pm$ 0	44.1 $\pm$ 6.3	27.9 $\pm$ 4.4
<i>Carlina vulgaris</i>	+	+	–	100 $\pm$ 0	83.0 $\pm$ 2.8	45.3 $\pm$ 3.8
<i>Centaurea stoebe</i>	+	+	+	100 $\pm$ 0	90.0 $\pm$ 4.9	40.0 $\pm$ 5.2
<i>Dianthus carthusianorum</i>	+	+	+	80.0 $\pm$ 2.1	45.5 $\pm$ 7.3	23.2 $\pm$ 3.8
<i>Euphrasia stricta</i>	+	+	–	26.7 $\pm$ 3.5	15.7 $\pm$ 6.8	4.1 $\pm$ 1.7
<i>Inula salicina</i>	+	+	+	100 $\pm$ 0	56.3 $\pm$ 3.2	29.7 $\pm$ 2.4
<i>Molinia coerulea</i>	+	+	+	100 $\pm$ 0	74.7 $\pm$ 4.4	58.5 $\pm$ 4.0
<i>Plantago media</i>	+	+	+	100 $\pm$ 0	72.1 $\pm$ 3.4	53.2 $\pm$ 3.7
<i>Prunella vulgaris</i>	+	+	–	100 $\pm$ 0	85.6 $\pm$ 2.8	51.3 $\pm$ 2.2
<i>Scabiosa ochroleuca</i>	+	+	+	100 $\pm$ 0	81.3 $\pm$ 6.6	42.1 $\pm$ 5.3
<i>Thymus pulegioides</i>	+	+	–	100 $\pm$ 0	86.3 $\pm$ 4.1	27.4 $\pm$ 4.3
<i>Tofieldia calyculata</i>	–	+	+	0.0	0.0	0.0
<i>Trifolium montanum</i>	+	+	–	100 $\pm$ 0	69.3 $\pm$ 5.7	43.6 $\pm$ 1.8
<i>Valeriana officinalis</i>	+	+	–	93.3 $\pm$ 3.3	23.3 $\pm$ 4.8	17.5 $\pm$ 5.7

Abbreviations:

AM – arbuscular mycorrhiza; A – arbuscules; V – vesicles; C – coils;

F% – mycorrhizal frequency; M% – intensity of root cortex colonization;

A% – arbuscule abundance

## DISCUSSION

The investigations of the mycorrhizal status of 20 plant species established on the soda heap in Jaworzno showed that 85% of them were associated with AMF of the phylum Glomeromycota. A similar proportion of plants with arbuscular mycorrhizae in the total number of plants examined was found by, e.g., Dodd et al. (2002) in a chalk marl reclamation platform created by the tunnelling of the Cannel Tunnel Railing in UK and by Pawłowska (1991) in a 32-year-old sedimentation basin with wastes from a soda factory. This confirms conclusions of many studies that AMF may tolerate very high pH and harmful concentrations of different soil chemical elements (e.g., Oliviera et al. 2005; Pawłowska 1991; Pawłowska et al. 1996; Turnau et al. 2001).

Of the plant species potentially able to associate with AMF, only roots of *Tofieldia calyculata* (Liliaceae) did not contain arbuscules (Table 3), although they hosted few coils and vesicles (Fig. 4) highly resembling those formed by fungi of the Glomeromycota. Arbuscules are the most important exponents of active arbuscular mycorrhizae (Smith and Read 1997). All investigations of the mycorrhizal status of *Tofieldia calyculata* so far performed in Poland showed this plant to not associate with AMF (Dominik and Nespiak 1953; Dominik et al. 1954a, b; Dominik and Pachlewski 1956; Nespiak 1953; Zubek et al. 2005). However, most of the three-quarters of the British species of the Liliaceae examined developed vesicular-arbuscular mycorrhizae, including a member of the genus *Tofieldia* (Harley and Harley 1987). *Tofieldia calyculata* is a species characteristic of calcareous fens of the Caricion davallianae Klika 1934 alliance (Matuszkiewicz 2001). Thus, both the Jaworzno soda heap and the other Polish sites in which the

mycorrhizae of this plant were examined (see above) were untypical for it. The real mycorrhizal status of *Tofieldia calyculata* existing in the conditions of the Jaworzno soda heap may reveal (1) studies of roots of this plant in different periods of vegetation, (2) cultivation of *Tofieldia calyculata* in pot cultures with its field-collected rhizosphere soils to initiate sporulation of associated AMF (Błaszowski et al. 2002; Stutz and Morton 1996), and the use of specific DNA primers exhibiting AMF hidden in roots of this plant (Kubota et al. 2005; Turnau et al. 2001).

The finding of arbuscular mycorrhizae in *Centaurea stoebe* and *Trifolium montanum* by the authors of this paper (Figs 2, 3, and 5-7) is the first report of the mycorrhizal status of these plant species.

Of the two *Epipactis* spp. examined, i.e., *Epipactis atrorubens* and *Epipactis palustris*, only the former harboured orchid mycorrhizae (Fig. 8), as e.g. Harley and Harley (1987) showed in Great Britain and Pawłowska (1991) when this plant grew in the Cracow (Poland) soda factory wastes. According to Harley and Harley (1987), all the species of the British flora investigated formed orchid mycorrhizae, including *Epipactis palustris*.

The presence of arbuscules in all plant species recognized to associate with AMF (Table 3) indicates that this symbiosis functioned in the soil conditions highly injurious for plants. Arbuscules are the main structures of exchange of nutrients between the two partners of this type of mycorrhizae (Smith and Read 1997). Another evidence of functioning of mycorrhizae in the conditions of the Jaworzno soda heap was the frequent occurrence of coils among the mycorrhizal structures revealed. Apart from arbuscules, coils may also serve as sites of carbon acquisition by the fungus and phosphorous efflux to the host plant (Smith and Smith 1996).

The species diversity of the spore populations extracted from field-collected substrate samples of the Jaworzno heap was relatively low and was limited to only five species of the genus *Glomus*. A similar species richness was found in many other studies involving heavy-metal polluted sites (Pawłowska et al. 1996), soils highly acidified and salted (Turnau et al. 2001), and other severely degraded and polluted ecosystems (e.g., Vosatka and Dodd 2002). Constraints in AMF community structure to only *Glomus* spp. have earlier frequently been observed in contaminated soils (Oliviera et al. 2005; Vosatka and Dodd 2002). However, many species of AMF sporulate seasonally or rarely in the field (Błaszczowski et al. 2002; Stutz and Morton 1996). Therefore, the recognition of the full species composition of AMF associated with plants of the Jaworzno soda heap will need to cultivate their rhizosphere soils in successive trap cultures and gradual extraction of spores of appearing fungi. Stutz and Morton (1996) showed that 75% of the species found after three culture cycles were not detected in the first trap culture. Nevertheless, as Turnau et al. (2001) suggested, the sporulation of the five *Glomus* spp. in the arduous site examined by the authors of this paper may indicate that only these quickly adapted to exist. *Glomus constrictum*, *G. fasciculatum*, *G. macrocarpum*, *G. microcarpum*, and *G. mosseae* occur in all regions of the world (Błaszczowski 2003). According to Stahl and Christensen (1982), a wide distribution of some species of AMF results from easiness of their genetic adaptation to different environmental conditions. Read (2002) concluded that disturbance, although causes losses of genetic diversity, also leads to selection of distinctive genotypes specially adapted to particular conditions.

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